COMPLEXATION OF NEW ACTIVE ANTIBACTERIAL ADAMANTAN DERIVATIVES WITH β CD: PREPARATION AND CHARACTERIZATION OF COMPLEXES STUDY OF THE THERMOTROPIC PROPERTIES OF PURE AND COMPLEX FORM WITH DIPALMITOYL PHOSPHATIDYLCHOLINE BILAYERS

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ABSTRACT

β-Cyclodextrin (βCD) complexes of I1-8, I1-10, I1-12, were prepared, isolated and characterized in solid and liquid form. Their thermotropic effects were studied by inserting them in DPPC bilayers both in pure and complexed forms. The results have shown that the presence of I1-8 causes spliting, broadening and lowering of the phase transition of DPPC bilayers. Their effects are more significant when I1-10 and I1-12 are inserted. These differential effects are eliminated when the above studied three molecules are incorporated in a complex form with βCD in DPPC bilayers. The obtained results suggest that the bromine salts in the bilayer are likely to remain in the complex form rather than released in the membrane.

INTRODUCTION

The microbiology of skin infections is changing. Thus, there is an increasing incidance of infections caused by strains, that are resistant to many antibiotics. The development of new potent topical preparations may change the traditional preference for systemic drugs in managing these disorders. The octyl, decyl and dodecylbromide salts of 2-(3-dimethylaminopropyl)-tricyclo [3.3.1.13,7]decyl-2-ol (I-1-8, I-1-10, I-1-12), were synthesized in our laboratory and showed an activity of MIC values ranging from $1x10^{-5}$ to $1x10^{-3}$ and limited aqueous solubility.
Although in recent literature a plethora of drug CD complexes have been prepared and studied for their solubility chemical stability and other physicochemical properties, studies on the interaction of this supramolecular assemblies with phospholipidic bilayers is rare.
The effectiveness of the quaternary ammonium salts appears to be a result of disturbance caused to the microbial cell membrane leading to leakage of intracellular compounds. At the same time, some microorganism possess impermeable cell membranes that prevent influx of the drug. Ohers lack the both case hydrophilic drug carrier such as CDs may facilitate the transversation of

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transport system that isequired for entrance of the drug into the bacterial cell. In this kind of outer membranes. The study of thermotropic properties of various drugs in a complex form with cyclodextrins in phospholipid bilayers is very useful because they give valuable information of their potential use as drug releasing agents.

2. MATERIALS and METHODS

2.1 Materials

The active compounds were synthesised and purified in our laboratory.. All other chemicals, were reagent grade and were used without further purification. βCD complexes of the active compounds has been prepared by using the precipitation method. Dipalmitoylphosphatidylcholine (DPPC) was obtained from Avanti Polar Lipids, Inc. AL, USA.

2.2 Methods

Drug-cyclodextrin interactions in aqueous solution were studied by Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR) and in solid state by Thermal Analysis (DSC). Appropriate amount of phospholipid and the bromine salts of quaternary dimethylamino adamantanol (or their complex with β-CD) were dissolved in spectroscopic grade chloroform and the two solutions mixed. The solvent was then evaporated by passing a stream of O_2-free nitrogen over the solution at 50 °C and the residue was placed under vacuum (0.1 mmHg) for 12 h.

Differential Scanning Calorimetry: The containing phospholipid prepared sample was hydrated (50% w/w) and was transferred to a stainless steel capsule (Perkin Elmer) and sealed hermetically. Thermograms were obtained on Perkin-Elmer DSC-7 instrument. All samples were scanned at least twice until identical thermograms were obtained using a scanning rate of 2.5 °C/min. Samples containing drug alone and mixed or complexed with β-CD were scanned using a scanning rate of 2,5 °C/min.

3. RESUTS AND DISCUSSION

3.1 Characterization of the complexes

The different thermotropic properties between the mixture and complexes is a result of their complexation.

3.2 Phosphatidylcholine bilayers

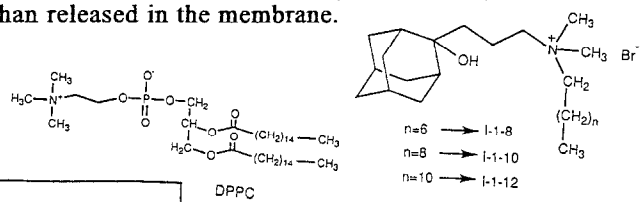
Certain hydrated phospholipids spontaneously form bilayers which share many of the conformational and dynamic properties of the natural membranes. Studies with these fully hydrated phospholipids are, therefore, useful since they allow us to gain insight into the physical chemistry of lipid interactions in natural membranes in which phospholipids are believed to exist largely as liquid crystalline bilayers. Among the membrane phospholipids, phosphatidylcholines are a major component and their phase properties have received a great deal of attention. The calorimetric measurements for a hydrated DPPC preparation show two endothermic transitions in the temperature range usually used to study membranes; a broad low-enthalpy pretransition (T'c=35.3 °C) and a main transition (Tc=41.2 °C) (Fig. 3, top). Below the pretransition, the phospholipid molecules are arranged in a one-dimensional lamellar gel phase ($L_{\beta'}$), while above the main transition they exist in the liquid crystalline phase (L_α). At temperatures between T'c and Tc, there is a ripple phase

($P_{\beta'}$) which on the basis of solid-state NMR evidence, has been shown to be composed of coexisting gel and liquid crystalline components.

3.3 Effects of drugs in phosphatidylcholine bilayers

The thermograms of DPPC with or without the active molecules are shown in Fig. 3. The presence of 0.10 molar ratio (x=0.10) I-1-8 in DPPC bilayers results in splitting and broadening of the phase transition as well as lower of the phase transition temperature of DPPC bilayers. The other two molecules I-1-10 and I-1-12 exert similar thermotropic effects in DPPC bilayers. Thus, the presence of either of the above two molecules lowers the phase transition and causes more significant broadening in the main phase transition temperature. A splitting was not observed in the presence of these two molecules depicting that they do not create any heterogeneity (domains) in the membrane bilayer as it happens with I-1-8 .

The thermograms of DPPC/(x=0.10)β-CD bilayers with or without either of the studied molecules are shown in Fig. 4. The presence of β-CD affects only marginally DPPC bilayers by lowering (ca. 1.0 °C) and splitting the top of the main phase transition. The presence of either comlexes with β-CD of the three molecules in the DPPC bilayers lowers the phase transition temperature and broadens the phase transition in a similar way. It appears, therefore, that the differential effects of the three studied compounds are eliminated when are incorporated in a complex form with β-CD in DPPC bilayers. The obtained results can be interpreted that the bromine salts in the bilayers are likely to remain in the complex form rather than released in the membrane.



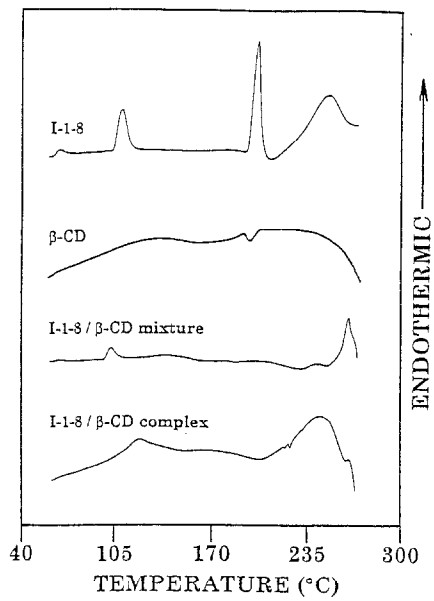


Figure 1: Characterization of the I-1-8 / β-CD complex

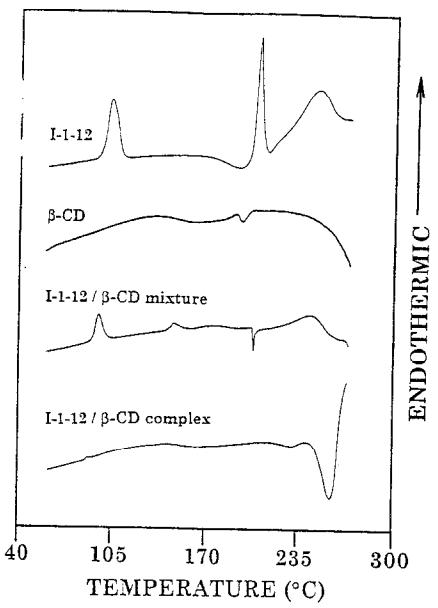


Figure 2: Characterization of the I-1-12 / β-CD complex

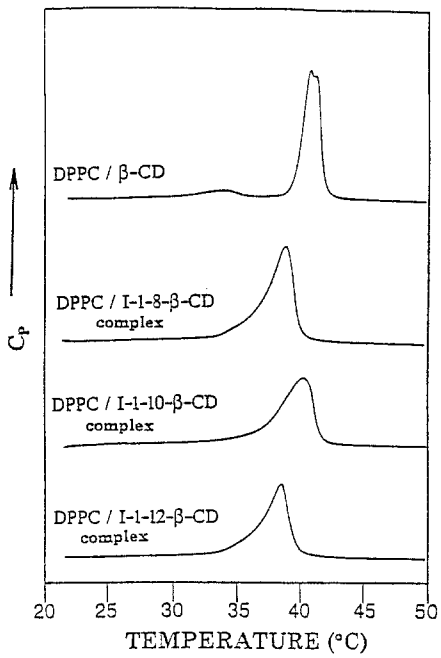


Figure 3

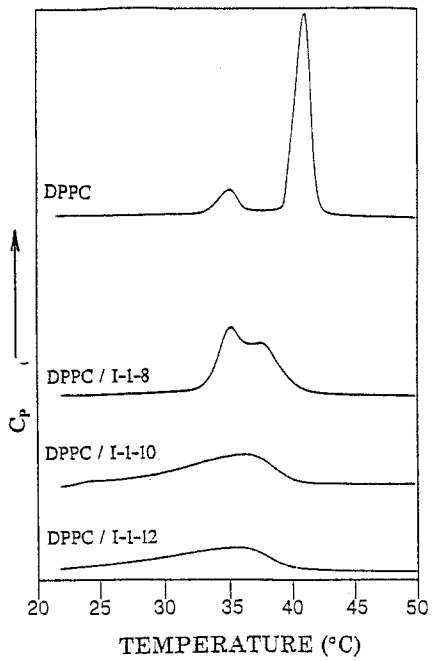


Figure 4

Figure 3: Normalized thermograms of DPPC, DPPC / (x=0.10) I-1-8, DPPC / (x=0.10) I-1-10 and DPPC (x=0.10) I-1-12.

Figure 4: Normalized thermograms of DPPC / β-CD, DPPC / (x=0.10) I-1-8-β-CD, DPPC / (x=0.10) I-1-10-β-CD and DPPC / (x=0.10) I-1-12-β-CD.

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